Temperature stress increases hybrid incompatibilities in the parasitic wasp genus Nasonia

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Protocol S1:

DNA extraction protocol Koevoets, van de Zande and Beukeboom, Temperature stress increases hybrid incompatibilities in the parasitic wasp genus *Nasonia.*

Adjusted after Hoarau *et al.* (2007) and Whitlock *et al.* (2008).

Males were ground with a sterile grinder in 96-wells titer plates. 100 µL digestion buffer (0.5% SDS, 100mM NaCl, 10 mM Tris:HCl pH 8.0, 25 mM EDTA and freshly added 0.2 µg/µL proteinase-K) was added to each sample and incubated over-night at 56°C (plate sealed with ThermalSeal). 40 µL 6 M NaCl and 140 µL $CHCl_3$ was added and mixed briefly. The titer plates were again sealed with a ThermalSeal and centrifuged in a Qiagen Sigma 4-15C centrifuge for 20 minutes at 3000 rpm. 60 µL of the water phase was transferred to a 96-wells fiber plate (Millipore: Multiscreen$_{HTS}$-FB plate), which was pre-wetted with 60 µL binding buffer (6 M NaI and 0.1M Na_2SO_3, clarified through standard filter paper, then through 0.45 µm filter). The plate was incubated at room temperature for 5 minutes, then centrifuged for 15 minutes at 1000 rpm, followed by 10 minutes at 2000 rpm. The flow-through was discarded, the DNA (attached to the filter plate) was washed with 60 µL ice-cold wash buffer [4.2 mL of a stock solution (20 mM Tris-HCl (pH 8), 1 mM EDTA, 0.1 M NaCl) diluted with 10.8 mL absolute ethanol per plate] and centrifuged for 10 minutes at 3000 rpm. The flow-through was discarded and the plate dried at room temperature for 30 minutes. Finally, the samples were eluted with 100 µL 56°C elution buffer (1 mM Tris-HCl (pH 8) and 0.1 mM EDTA), incubated for 5 minutes at room temperature and the plate was centrifuged for 5 minutes at 1000 rpm and 5 minutes at 2000 rpm, collecting the DNA in clean 96-wells titer plates. In case of failing DNA extraction (2 out of 10 plates using an earlier stage of the protocol, the protocol as described here, should minimize these failures), the remaining aqueous phase after the $CHCl_3$ step was removed. On this, the regular high salt-chloroform protocol was followed (Maniatis *et al.*, 1982). DNA extracted using the fiber plates was used for PCR amplification undiluted, while DNA extracted using the high salt-chloroform protocol was diluted 10x.

Hoarau G., Coyer J.A., Stam W.T. & Olsen J.L. 2007. A fast and inexpensive DNA extraction/purification protocol for brown macroalgae. *Mol. Ecol. Notes* 7: 191-193.

Maniatis T., Fritsch E.F. & Sambrook J. 1982. *Molecular cloning: a laboratory manual,* 11th edn. Cold spring harbor laboratory press, New York.

Whitlock R., Hipperson H., Mannarelli M. & Burke T. 2008. A high-throughput protocol for extracting high-purity genomic DNA from plants and animals. *Mol. Ecol. Resour.* 8: 736-741.